SECTION: Innovative Techniques and Technology, Gary N. Foulks, MD, Editor

TITLE: Less Invasive Corneal Transepithelial Electrical Resistance Measurement Method

AUTHORS: Masafumi Uematsu, MD, PhD,[1] Yasser Helmy Mohamed, MD,PhD,[1,2] Naoko Onizuka, MD, PhD,[1] Ryotaro Ueki, MD,[1] Daisuke Inoue, MD,[1] Azusa Fujikawa, MD, PhD,[1] Hitoshi Sasaki, PhD,[3] and Takashi Kitaoka, MD, PhD[1]

SHORT TITLE: Corneal Transepithelial Electrical Resistance Measurement/Uematsu et al

FOOTNOTES

Accepted for publication August 2015.

[1]Department of Ophthalmology and Visual Sciences, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan, [2]Department of Ophthalmology, EL-Minia University Hospital, EL-Minia, Egypt, and [3]Department of Hospital Pharmacy, Nagasaki University Hospital of Medicine and Dentistry, Nagasaki, Japan

Sources of support: None

The authors have no commercial or proprietary interest in any concept or product discussed in this article.

Single-copy reprint requests to: Masafumi Uematsu, MD, PhD (address below).

Corresponding author: Masafumi Uematsu, MD, PhD, Department of Ophthalmology and Visual Sciences, Graduate School of Biomedical Sciences, Nagasaki University, 1-7-1 Sakamoto, Nagasaki, Nagasaki 852-8501, Japan. Tel: +81-95-819-7345. Fax: +81-95-819-7347. E-mail: uematsu1124@outlook.jp

ABSTRACT

Purpose: To evaluate acute corneal permeability changes after instillation of benzalkonium chloride (BAC) using a newly developed in vivo less invasive corneal TER measurement method in animals and humans. Methods: We previously developed an in vivo method for measuring corneal transepithelial electrical resistance (TER) using intraocular electrodes in animals. This method can be used to precisely measure the decline of the corneal barrier function after instillation of BAC. To lessen the invasiveness of that procedure, we further refined the method for measuring the corneal TER by developing electrodes that could be placed on the surface of the cornea and in the conjunctival sac instead of inserting them into the anterior chamber. Corneal TER changes before and after exposure to 0.02% BAC were determined in this study using the new device in both rabbits and humans. Results: There was a significant decrease in the corneal TER after exposure of the cornea to 0.02% BAC solution in both rabbits and humans ($P<.01$). The results of this new less invasive method agreed with those of formerly established anterior chamber methods as regards rabbits experiment and was expected and satisfactory as regards human experiment.

Conclusion: This new less invasive corneal TER measurement method enables us for the first time to measure TER of the human cornea, allowing safe and reliable investigation of the direct effect of different eye drops treatments on the corneal epithelium.

KEY WORDS　benzalkonium chloride, cornea, transepithelial electrical resistance

Outline

I. Introduction

The cornea is one of the few human tissues that is always in direct contact with the environment. This together with its transparency makes the cornea a very special tissue. In particular, the corneal epithelium, which is the outer part of the cornea, acts as a barrier against the daily insults of the external environment. To ensure its transparency, the cornea does not have blood vessels for its nourishment. Nutrients are supplied by diffusion through the epithelium and endothelium layer, ensuring a proper homeostasis.[1] Corneal epithelium

tight junctions are the most apical intercellular junctions, and play an important role in the establishment and maintenance of the barrier function.[2,3] Disruption of corneal epithelial barrier function results in ocular irritation[4,5] and increased risk for microbial infections.[6]

The electrophysical properties of a cell or tissue can be determined by passing an electric current through the cell or tissue and measuring the voltage drop and potential difference across the tissue. When the current delivered and the voltage measured are known, the resistance of the tissue can be calculated using Ohm's Law: resistance (Ω) is equal to the voltage (V) divided by the current (I in amperes).[7]

Most ophthalmic drugs contain adjuvants such as surfactants and preservatives. They are often essential for ocular liquid formulations, solubilizing drugs, and preventing microbial contamination. Some of these adjuvants act as ocular penetrating enhancers, promoting drug penetration through the strong corneal barrier and modifying the physiochemical property of drugs.[8,9] At the same time, however, they damage the corneal epithelial structure, especially the transcellular integration of superficial cells, which is mainly maintained by tight junctions.[10,11] Therefore, investigation of the effect on the cornea of ophthalmic drugs and adjuvants is important.

Corneal transepithelial electrical resistance (**TER**) is maintained by corneal superficial cells with tight junctions between them, which together function as a corneal barrier that is highly resistant against invasion. Measuring changes in TER allows the quantitative and continuous evaluation of the state of the corneal epithelium and its barrier function. The method is used for electrophysiologically evaluating corneal toxicity induced by ophthalmic drugs.[12,13]

Measurement of corneal TER is a suitable method for evaluating corneal permeability and irritation quantitatively and continuously. TER reflects the barrier function of the epithelium. Lower corneal TER means more electrical current penetrates through the

damaged superficial cells and tight junctions between them. In addition, it is reported to be a very sensitive test for measuring electrical properties of the cornea.[14,15]

In vitro experimental systems using cultured cells are often employed to evaluate the barrier function of the corneal epithelium as well as injuries of the corneal epithelium caused by various drugs.[7,16,17] These experimental systems provide an excellent means of objectively comparing the potential for corneal injury among several ophthalmic solutions; however, the extent to which these experimental models reflect the condition of the eyes in vivo remains unclear.[18]

Only a few in vivo studies of corneal electrical properties have been reported, [19-21]due to the difficulty of performing such analyses. Recent approaches to performing in vivo measurements have adapted the existing TER measurement methods for use in living animals.[7,22]

We developed a new method of measuring the TER of live rabbit cornea. In this method, the cornea is not damaged by the experimental procedure and the TER is stable before drug administration. To measure corneal TER, we used a volt-ohm meter which generates ± 20 μA AC square wave current at 12.5 Hz. Therefore, it could measure TER every 0.08 s. In addition, TER was monitored with a recorder, which shows TER changes continuously. To our knowledge, no other study has evaluated acute corneal change within one second in vivo.[23]

However, the invasiveness of this procedure precludes its use in clinical practice. In order to overcome this problem, we recently developed a less invasive corneal TER measurement method.[24]

In previous studies, after developing a new in vivo method of measuring the TER of rabbit corneas, we demonstrated that benzalkonium chloride (**BAC**) concentrations between 0.005% and 0.02% immediately caused acute corneal barrier dysfunction.[23-25] The purpose of

this study was to evaluate acute corneal permeability changes after instillation of BAC using a new less invasive in vivo TER measurement method in animals and humans.

II. Materials and Methods

A. Chemicals

Ca^{2+}- and Mg^{2+}-free Hank's Balanced Salt Solution (**HBSS**) was obtained from Invitrogen Corporation (Carlsbad, CA, USA). BAC 10% solution (mixed BAC) was obtained from Wako Pure Chemical, Co, (Osaka, Japan).

B. Experimental Animals

Male white Japanese rabbits (KBT Oriental, Tosu, Japan) weighing 2.5 -3.0 kg were individually housed in cages under a controlled temperature (21°C) and humidity (50 ± 5%) and a 12:12 h light/dark cycle at the Laboratory Animal Center for Biomedical Research, Nagasaki University School of Medicine. The study was initiated when the rabbits reached weights of 3.0-4.0 kg, as this was the point where the corneal diameters were of suitable size for experimentation. The rabbits were treated in compliance with the ARVO Statement for the

Use of Animals in Ophthalmic and Vision Research.

C. Corneal TER Measurement In Vivo in Rabbits

The rabbits were anesthetized with an intramuscular injection of 30 mg/kg ketamine (Ketalar, Sankyo, Tokyo, Japan) and 5 mg/kg xylazine (Celactal, Bayer HealthCare, Osaka, Japan). A 1.0-mm diameter custom-made Ag/AgCl electrode (Physiotech, Tokyo, Japan) was placed within the tear fluid in the conjunctival sac. The pathway of the electrical current is shown in Figure 1. A 6.0 mm internal diameter (0.28 cm^2 inner area) nitrile rubber O-ring (Union Packing, SAN-EI, Osaka, Japan) was fixed on the cornea using biomedical adhesive (Alon-Alpha A, Sankyo, Tokyo, Japan). Subsequently, 80 μL of HBSS was placed inside the ring, with the second electrode then placed in HBSS on the cornea. In a period of just a few seconds, 1 mL of the HBSS as a control and 0.02% BAC were gently poured into the ring, with overflow aspirated. After an exposure period of 30seconds, the rings were washed out with 1 mL of HBSS.

The TER was measured in real time, using a volt-ohm meter (EVOMX, World Precision Instruments, Sarasota, FL, USA) that generates a ± 20 μA AC square wave current at12.5Hz. In the preliminary examination, we determined that the electrical resistance between electrodes without corneal epithelium is 222 Ω cm^2. Therefore, the TER value was defined as "measured TER-222"Ω cm^2. After obtaining the TER of the cornea before and after the exposure, results were then calculated as a percentage of the pre-exposure TER value (100%). The sample size for the corneal TER study was set at 3 to 4, which we found to be sufficient for our statistical analyses in our previous TER studies. [23- 26]

D. Corneal Transepithelial Electrical Resistance Measurement in Humans

A total of 6 eyes of 3 healthy volunteers were examined. The age of the participants was

29.3 ± 5.7 years (mean ± SD; age range, 23–34 years). Eyes with obvious ocular disorders were not included in this study. Written informed consent was obtained from all the subjects. The research protocol followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Nagasaki University Graduate School of Biomedical Sciences (number 10100131).

We used the same technique of TER evaluation as was used in rabbits with minor changes. Surface anesthesia was added to ocular surface using oxybuprocaine hydrochloride, which in the preliminary examination showed no effect on the corneal TER. The first electrode was placed in the conjunctival fornix, and the other was placed in HBSS on the corneal ring. The corneal ring was fixed in place by using eye ointment instead of glue to prevent any epithelial damage. The ointment was spread evenly on the under surface of the 6 mm-internal diameter of nitrile rubber O-ring. Then the ring was gently placed on the cornea. The electrodes on the cornea and in the conjunctival sac are shown in Figure 2. One mL of the HBSS as a control and 0.02% BAC were gently poured into the ring, with overflow aspirated. After an exposure period of 30 seconds, the rings were washed out using 1 mL of HBSS. After obtaining the TER of the cornea before and after the exposure, results were calculated as a percentage of the pre-exposure TER value (100%).

E. Slit Lamp Biomicroscopy Observation

Human corneas before and after the corneal TER measurement were examined by slit lamp biomicroscopy with fluorescein staining.

F. Statistical Analysis

All results were expressed as the mean ± standard error. Statistical comparisons were performed using Student's paired t-test for the TER measurements. Values of $P<0.01$ were

considered to indicate statistical significance.

III. Results

A. Corneal Transepithelial Electrical Resistance Measurement In Vivo in Rabbits

The mean corneal TER for the live rabbits used in this study was 750 ± 111 Ω cm^2. The TER decreased to 187 ± 11 Ω cm^2 following treatment with 0.02% BAC. There was a significant decrease in the corneal TER after exposure of the cornea to 0.02% BAC solution (P<.01). Figure 3 shows the TER changes that occurred after corneal exposure to 0.02% BAC.

B. Corneal Transepithelial Electrical Resistance Measurement in Humans

The mean corneal TER for the humans used in this study was 690 ± 69 Ω cm^2. The TER decreased to 259 ± 27 Ω cm^2 following treatment with 0.02% BAC. There was a significant decrease in the corneal TER after exposure of the cornea to 0.02% BAC solution (P<.01). Figure 3 shows the TER changes that occurred after corneal exposure to 0.02% BAC. The time courses of the corneal TER change in humans and rabbits are shown in Figure 4.

C. Slit Lamp Biomicroscopy Observation

There was no change or slight superficial punctate keratopathy after corneal TER measurement in humans (Figure 5).

IV. Discussion

Many methods have been used to evaluate corneal irritation and permeability induced by ophthalmic drugs. Ocular irritation is conventionally tested according to modified procedure of Draize by scoring the degree of damage to rabbit eyes.[27,28] Alternative methods include evaluation of toxicity in cultured ocular cells,[29] direct confocal microscopic analysis,[30] and various other approaches using isolated animal corneas.[31,32] Corneal drug permeability has been evaluated by diffusion experiments in vitro.[9] The epithelial barrier function in humans has been examined by measuring the permeability of fluorescence.[33-35]

Drug toxicity must be rapidly evaluated because topically instilled drugs become rapidly diluted with tears.[36] However, ocular surface changes are difficult to elicit within a short period using the previous described methods.

Because TER is a sensitive, reliable, and versatile test of barrier function, it is used to study the integrity of tissues and cell sheets, such as the intestinal epithelium and Madin–Darby canine kidney cells.[37–39] Furthermore, TER is also a useful indicator of corneal toxicity.[15, 40, 41]

In general, TER reflects the barrier function of epithelium, with lower corneal TER values indicative of the penetration of greater amounts of electrical current through the damaged superficial cells and tight junctions existing in the epithelium. The corneal toxicity of ophthalmic drugs was investigated by measuring TER in isolated corneas in vitro. However, the isolated cornea is difficult to handle because it can easily become damaged during experimental procedures and by hydration with buffers, and the TER can be unstable at the start of experiments.

We previously described a novel corneal TER measurement system in vivo using custom-designed thin stick electrodes and a volt-ohm meter to measure the barrier function of the intact cornea in rabbits. This design more accurately reflects the clinical instillation of

ophthalmic drugs and provides relevant data about the acute corneal toxicity of some eye drops.[23-26] Although that previously described corneal TER measurement system in vivo was informative and reliable, it was still invasive and could not be used clinically for human studies because of intraocular electrodes. So, we developed this new less invasive corneal TER measurement method in which one electrode is placed on the surface fluid of the cornea and the other one in the conjunctival fornix.

Ophthalmic solutions are used not only to treat ocular surface disorders but also intraocular diseases such as glaucoma. Preservatives are a major component of the ophthalmic preparations, providing antimicrobial activity and preventing decomposition of the active drug in multidose bottle. As a soluble antimicrobial agent and surfactant, BAC is the most commonly used preservative in ophthalmic solutions because of its apparently good safety/efficacy profile.[42]

BAC is invasive to the cornea. Its effect is so strong that it destroys not only the surface of corneal superficial cells but also the tight junctions between them. Corneal TER mainly involves electrical resistance of superficial cell membranes and tight junction; therefore, destruction of the corneal surface leads to increased current permeability through the cornea, which is represented as decreased TER.[23]

In our previous studies, after developing a new in vivo method of measuring the corneal TER of rabbit corneas, we demonstrated that 0.02% BAC concentration immediately caused acute corneal barrier dysfunction.[23-26] In this study, we checked the reliability and the efficacy of this new less invasive corneal TER measurement method by measuring the corneal TER changes after application of 0.02% BAC in both rabbits and humans. The results of this new less invasive method agreed with those of the formerly established anterior chamber method used in rabbit experiments.

In the new method, the cornea is intact without being excised, it is not soaked in

buffer solution for a long time, and TER is stable before drug administration. As the concentration of the drug after topical instillation decreases rapidly with the tears in the conjunctival sac and with additional tear secretion induced by drug stimulation, it is important to evaluate the effect of agents in a short period, preferably within 1 min after drug instillation. In this study, as in the previous one, TER was measured by a volt-ohm meter every 0.08 s and monitored continuously with a recorder. Therefore, the time course of TER change in a short period was clearly observed. Thus, the method maintains the advantage of the previous device in determination of acute corneal TER changes after application of eye solutions.

Although the corneal epithelium under the ring may be affected by ointment in the human study, we confirmed in the preparatory experiment that TER findings with ointment was the same as those with petroleum jelly (Vaseline) used for rubber ring fixation. Petroleum jelly, which is used clinically as an ophthalmic ointment, is not invasive to the corneal epithelium.[43]

The only concern about this less invasive technique, in our view, is the variation in TER seen before treatment. This problem also existed with the previous invasive technique. Variation of TER before treatment should be measured as an individual difference between corneas. Thus, to produce reliable data, the conditions of the experimental setting need to be identical, and TER changes should be expressed in percentages.

V. Conclusion

Our new less invasive corneal TER measurement method enables us for the first time to measure TER of the human cornea, allowing safe and reliable investigation of the direct effect of different eye drops on the corneal epithelium.

References:

1. Kinoshita S, Adachi W, Sotozono C, et al. Characteristics of the human ocular surface epithelium. *Prog Retin Eye Res* 2001;20:639–73

2. Vandenbrouche E, Mehta D, Minshall R, Malik AB. Regulation of endothelial junctional permeability. *Ann N Y Acad Sci* 2008;1123:134–45

3. Miyoshi J, Takai Y. Structural and functional associations of apical junctions with cytoskeleton. *Biochim Biophys Acta* 2008;1778:670–91

4. Yokoi N, Kinoshita S. Clinical evaluation of corneal epithelial barrier function with the slit-lamp fluorophotometer. *Cornea* 1995;14:485–9

5. Pflugfelder SC, Farley W, Chen LZ, et al. Matrix metalloproteinase-9 knockout confers resistance to corneal epithelial barrier disruption in experimental dry eye. *Am J Pathol* 2005;166:61–71

6. Alarcon I, Tam C, Mun JJ, et al. Factors impacting corneal epithelial barrier function against Pseudomonas aeruginosa traversal. *Invest Ophthalmol Vis Sci* 2011;52: 1368–77

7. Fukuda M, Sasaki H. Quantitative evaluation of corneal epithelial injury caused by n-heptanol using a corneal resistance measuring device in vivo. *Clin Ophthalmol* 2012;6:585-93

8. Saettone MF, Chetoni P, Cerbai R, et al. Evaluation of ocular permeation enhancers: in vitro effects on corneal transport of four β blockers, and in vitro/in vivo toxic activity. *Int J Pharm* 1996;142:103–13

9. Sasaki H, Yamamura K, Mukai T, et al: Enhancement of ocular drug penetration. *Crit Rev Ther Drug Carrier Syst* 1999;16: 85–146

10. Tonjum AM: Effects of benzalkonium chloride upon the corneal epithelium studied with scanning electron microscopy. *Acta Ophthalmol* 1975;53:358–66

11. Burstein NL: Preservative cytotoxic threshold for benzalkonium chloride and

chlorhexidine digluconate in cat and rabbit corneas. *Invest Ophthalmol Vis Sci* 1980;19:308–13

12. Potts AM, Modrell RW: The transcorneal potential. *Am J Ophthalmol* 1957;44: 284–90

13. Donn A, Maurice DM, Mills NL. Studies on the living cornea in vitro. I. Method and physiologic measurements. *Arch Ophthalmol* 1959;62:741–47

14. Nakashima M, Nakamura T, Teshima M, et al. Breakdown evaluation of corneal epithelial barrier caused by antiallergic eyedrops using an electrophysiologic method. *J Ocul Pharmacol Ther* 2008;24:43-51

15. Chetoni P, Burgalassi S, Monti D, et al. Ocular toxicity of some corneal penetration enhancers evaluated by electrophysiology measurements on isolated rabbit corneas. *Toxicol In Vitro* 2003;17:497-504

16. Pellinen P, Lokkila J. Corneal penetration into rabbit aqueous humor is comparable between preserved and preservative-free tafluprost. *Ophthalmic Res* 2009;41:118–22

17. Yee RW, Norcom EG, Zhao XC. Comparison of the relative toxicity of travoprost 0.004% without benzalkonium chloride and latanoprost 0.005% in an immortalized human cornea epithelial cell culture system. *Adv Ther* 2006;23:511–19

18. Denoyer A, Ossant F, Arbeille B. Very-high-frequency ultrasound corneal imaging as a new tool for early diagnosis of ocular surface toxicity in rabbits treated with a preserved glaucoma drug. *Ophthalmic Res* 2008;40:298–308

19. Biermann H, Boden K, Reim M. Measuring electrical impedance in normal and pathologic corneas. *Fortschr Ophthalmol* 1991;88:17–20

20. Jurgens I, Rosell J, Riu PJ. Electrical impedance tomography of the eye: In vitro measurements of the cornea and the lens. *Physiol Meas* 1996;17:187–95

21. Watanabe M, Mokudai Y, Ueno H, et al. Dielectric measurements on the rabbit

cornea using a surface electrode. *Nippon Ganka Gakkai Zasshi* 1993;97:569–74

22. Chen W, Hu J, Zhang Z, et al. Localization and expression of zonula occludins-1 in the rabbit corneal epithelium following exposure to benzalkonium chloride. PLoS One. 2012;7:e40893

23. Uematsu M, Kumagami T, Kusano M, et al. Acute corneal epithelial change after instillation of benzalkonium chloride evaluated using a newly developed in vivo corneal transepithelial electrical resistance measurement method. *Ophthalmic Res* 2007;39:308-14

24. Uematsu M, Mohamed YH, Onizuka N, et al. A novel in vivo corneal trans-epithelial electrical resistance measurement device. J Pharmacol Toxicol Methods 2015;76:65-71

25. Kusano M, Uematsu M, Kumagami T, et al. Evaluation of acute corneal barrier change induced by topically applied preservatives using corneal transepithelial electrical resistance in vivo. *Cornea* 2010;29:80-5

26. Onizuka N, Uematsu M, Kusano M, et al. Influence of different additives and their concentrations on corneal toxicity and antimicrobial effect of benzalkonium chloride. *Cornea* 2014;33:521-6.

27. Draize JH, Woodard G, Calvery HO. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J Pharmacol Exp Ther* 1944;82:377-90

28. York M, Steiling W. A critical review of the assessment of eye irritation potential using the Draize rabbit eye test. *J Appl Toxicol* 1998;18:233-40

29. Kruszewski FH, Walker TL, DiPasquale LC. Evaluation of a human corneal epithelial cell line as an in vitro model for assessing ocular irritation. *Fundam Appl Toxicol* 1997;36:130-40

30. Furrer P, Plazonnet B, Mayer JM, et al. Application of in vivo confocal microscopy

to the objective evaluation of ocular irritation induced by surfactants. *Int J Pharm* 2000;207:89-98

31. Prinsen MK. The chicken enucleated eye test (CEET): a practical (pre)screen for the assessment of eye irritation/corrosion potential of test materials. *Food Chem Toxicol* 1996;34:291-6

32. Monti D, Chetoni P, Burgalassi S, et al. Increased corneal hydration induced by potential ocular penetration enhancers: assessment by differential scanning calorimetry (DSC) and by desiccation. *Int J Pharm* 2002;232:139-47

33. Göbbels M, Spitznas M. Influence of artificial tears on corneal epithelium in dry-eye syndrome. *Graefes Arch Clin Exp Ophthalmol* 1989;227:139-41

34. Joshi A, Maurice D, Paugh JR. A new method for determining corneal epithelial barrier to fluorescein in humans. *Invest Ophthalmol Vis Sci* 1996;37:1008-16

35. Ishibashi T, Yokoi N, Kinoshita S. Comparison of the short-term effects on the human corneal surface of topical timolol maleate with and without benzalkonium chloride. *J Glaucoma* 2003;12:486-90

36. Jordan A, Baum J. Basic tear flow. Does it exist? *Ophthalmology* 1980;87:920-30

37. Moyes SM, Smyth SH, Shipman A, et al. Parameters influencing intestinal epithelial permeability and microparticle uptake in vitro. *Int J Pharm* 2007;337:133–41

38. Blikslager AT, Moeser AJ, Gookin JL, et al. Restoration of barrier function in injured intestinal mucosa. *Physiol Rev* 2007;87:545–64

39. Balda MS, Whitney JA, Flores C, et al. Functional dissociation of paracellular permeability and transepithelial electrical resistance and disruption of the apical-basolateral intramembrane diffusion barrier by expression of a mutant tight junction membrane protein. *J Cell Biol* 1996;134:1031–49

40. Rojanasakul Y, Liaw J, Robinson JR. Mechanisms of action of some penetration

enhancers in the cornea: laser scanning confocal microscopic and electrophysiology studies. *Int J Pharm* 1990;66:131–42

41. Rojanasakul Y, Robinson JR. Electrophysiological and ultrastructural characterization of the cornea during in vitro perfusion. *Int J Pharm* 1990;63:1–16

42. Baudouin C, Labbe´ A, Liang H, et al. Preservatives in eyedrops: The good, the bad and the ugly. *Prog Retin Eye Res* 2010;29:312–4

43. Hanna C, Fraunfelder FT, Cable M, Hardberger RE: The effect of ophthalmic ointments on corneal wound healing. *Am J Ophthalmol* 1973;76:193–200

Figure Legends

Figure 1.The pathway of the electrical current. Electrical resistance except corneal epithelium is 222 Ω cm^2.

Figure 2. Corneal TER measurement in human. The electrodes are placed in the HBSS on the cornea and in the conjunctival sac.

Figure 3. The normal corneal TER value is 690 ± 69 Ω cm^2 for humans and 750 ± 111 Ω cm^2 for rabbits. Following 30 seconds treatment with 0.02% BAC, the TER significantly decreased in humans (*: P <.01, 259 ± 27 Ω cm^2) and in rabbits († : P <0.01, 187 ± 11 Ω cm^2) compared with the normal TER values.

Figure 4. The time courses of the TER during exposure to 0.02% BAC. The TER decreased in almost the same manner in humans and rabbits.

Figure 5. Slit lamp biomicroscopic observation before (a) and after (b) corneal TER measurement in humans. There was no change or slight superficial punctate keratopathy on the cornea after TER measurement.

Figure 1.

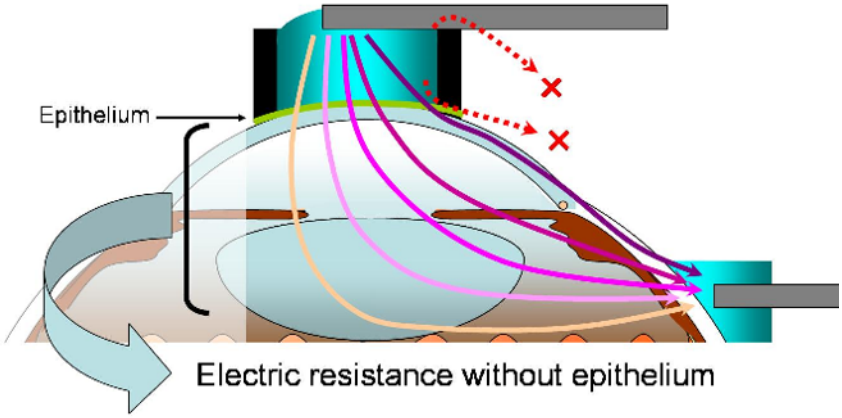

Figure 2.

Figure 3.

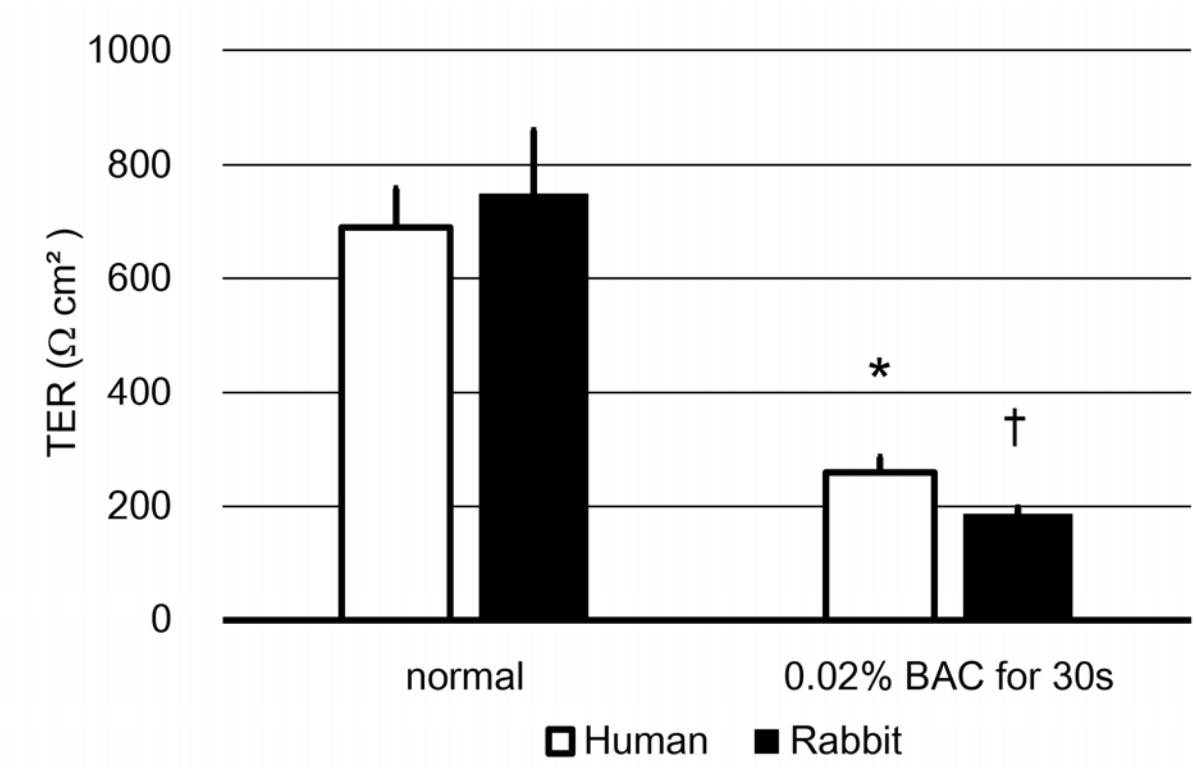

Figure 4.

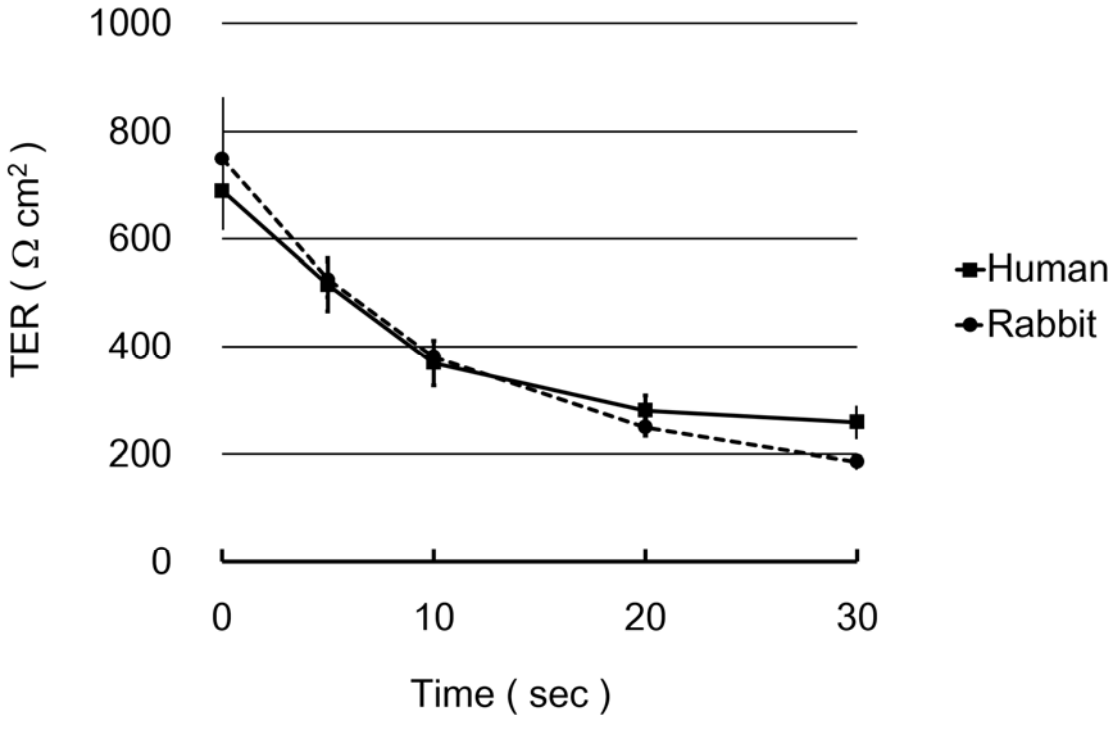

Figure 5.